Activities of Respiratory Enzymes During the Metamorphosis of the Face Fly, *Musca autumnalis* De Geer[1]

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Abstract: The activities of alcohol, succinic, malic, glucose, glutamic, alpha-glycerophosphate, lactic, and isocitric dehydrogenases, the malic enzyme, and cytochrome oxidase were determined during the metamorphosis of the face fly, *Musca autumnalis*.

Total alpha-glycerophosphate, alcohol, malic, and succinic dehydrogenases as well as the malic enzyme exhibited U-shaped activity. Greatest activity was shown by the malic dehydrogenase. Isocitric dehydrogenase activity was high initially and remained high until the 2-day pupa, and thereafter showed a progressive decline. Glucose dehydrogenase activity was low and remained fairly steady during the entire pupal stage. Alcohol dehydrogenase decreased steadily during the first days of metamorphosis, reached a low value on the third day, and then increased to reach its highest value in the adult stage. Succinic dehydrogenase exhibited a similar pattern, but the level of activity was not as high as most of the other dehydrogenases. Glutamic dehydrogenase showed low activity in the larval stage. It decreased during the first several days of the pupal life and completely disappeared by the fourth day. The activity of lactic dehydrogenase was very low throughout metamorphosis. Malic enzyme exhibited high activity in the larva, prepupa, and again in the adult stage. Cytochrome oxidase activity was also U-shaped during metamorphosis.

The O_2 consumption of holometabolous insects follows a U-shaped curve during metamorphosis. This phenomenon was first described by Krogh (1914) for the mealworm, *Tenebrio molitor*, and subsequently has been confirmed by the following investigators employing a variety of insect species: Clare, 1925; Fink, 1925; Bodine and Orr, 1925; Ludwig, 1931; Dobzhansky and Poulson, 1935; Wolsky, 1938; Sacktor, 1951; Ito, 1954; Cotty, 1956; and Ludwig and Barsa, 1956.

Since the causative factors responsible for the U-shaped respiratory curve are not fully understood, various explanations have been advanced. Krogh (1914) and Fink (1925) believed the changes in O_2 consumption to be associated with different degrees of tissue organization. The activity of cytochrome oxidase has been investigated as a rate-limiting factor in respiratory metabolism. Wolsky (1938), Williams (1950), Ludwig (1953) and Diamantis (1962) found U-shaped activity curves for cytochrome oxidase during the pupal stages of the fruit fly *Drosophila melanogaster*, the moth *Platysamia cecropia*, the Japanese beetle *Popillia japonica*, and the flour moth *Ephestia kühniella*, respectively. A correlation between succinic dehydrogenase activity and respiratory metabolism has been described by Wolsky (1941) for *Drosophila melanogaster*, Ito (1954) for *Bombyx mori*, Ludwig and Barsa (1955) for *Popillia japonica* and for *Tenebrio*

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molitor (1958). Agrell (1949) described total dehydrogenase activity and the activities of malic, citric, and glutamic dehydrogenases as U-shaped during the metamorphosis of the blow fly, *Calliphora erythrocephala*. Ludwig and Barsa (1958) found malic and succinic dehydrogenases and the malic enzyme activities to be U-shaped during the metamorphosis of *Tenebrio molitor*. In 1959, they found that with the house fly alcohol and alpha-glycerophosphate dehydrogenases also followed U-shaped curves. Diamantis (1962) described similar activity for alpha-glycerophosphate I and II, malic, isocitric and succinic dehydrogenases and the malic enzyme. His report of the U-shaped activity of isocitric dehydrogenase is at variance with the findings of Ludwig and Barsa (1959) for the house fly. They reported the isocitric dehydrogenase showed a steady decrease during metamorphosis. Diamantis (1962) also found low glutamic dehydrogenase activity at all stages, whereas Ludwig and Barsa (1959) found that it disappeared early in the pupal stage.

In the present investigation a study was made of cytochrome oxidase and the various dehydrogenases during the metamorphosis of the face fly *Musca autumnalis*.

MATERIALS AND METHODS

The insects used in this study were obtained from the United States Department of Agriculture Research Center, Beltsville, Maryland. They were reared in screened cages measuring 30 × 30 × 30 inches. The temperature of the rearing room was 25 ± 2°C and the relative humidity varied between 35–60 per cent. The light source consisted of two 160-W General Electric F 40 CW fluorescent lamps that gave a light intensity of approximately 150 ft-c measured at the top of the cages. The optimum photoperiod was found to be 16 hours extending from 6 a.m. to 10 p.m.

A mixture of skimmed milk and 5 per cent sucrose solution in a 2:1 ratio was placed daily in a petri dish containing a centrally located piece of absorbent cotton which served as a resting place for the flies when they were feeding. Approximately 10 ml of citrated bovine blood was placed in a second dish and 3 ml of 5 per cent maltose solution was also added to this receptacle. Fresh cow dung was placed in a third dish to serve both as a source of food and as an oviposition medium. Each day after being removed from the cages, the dishes of manure were set aside for 48 hours and then examined for the presence of larvae. If larvae were found, the manure was transferred to a porcelain tray (15 × 10 × 3 inches) containing a large central mass of dung surrounded by a fairly thick layer of vermiculite into which the larvae migrated just prior to pupation. These trays were covered with a layer of cheesecloth and placed on shelves in the rearing room. Following pupation, the insects were gently removed to a small dish which was put in one of the rearing cages to await emergence.

The activities of alcohol, succinic, malic, glucose, glutamic, alpha-glycerophosphate, lactic, and isocitric dehydrogenases and the malic enzyme were determined by the Thunberg method as given by Umbreit, Burris and Stauffer (1957, p. 130). The insects were washed in an alcohol solution, according to the procedure followed by Cotty (1956) to remove surface bacteria before homogenization. Insects were homogenized by means of a motor-driven glass homogenizer for 1 minute in 0.03 M phosphate buffer, except in the case of isocitric dehydrogenase, where veronal buffer was used since the phosphate ion interferes with the activity of this enzyme. The buffers were adjusted to a pH of 7.4. A 3 per cent homogenate (1 ml) was incubated at 30°C for 30 minutes, and when NAD or NADP was used, the homogenate was pre-incubated with 0.5 ml of 0.2 per cent NAD or with 0.5 ml of 0.1 per cent NADP to oxidize the endogenous substrate. The smaller concentration of NADP was used because the addition of larger amounts did not increase enzyme activity. The homogenate was then placed in the side arm cap of the Thunberg tube. In the body of the tube were placed 1 ml of 1/10,000 per cent methylene blue, 1 ml of substrate (0.004 M), and a sufficient amount of buffer to bring the final volume to 6 ml. In measuring the activity of malic dehydrogenase, 0.5 ml of 0.24 M KCN was added to prevent inhibition by the oxalacetate formed (Green 1936). In determining the succinic dehydrogenase activity, 0.5 ml of a mixture of 0.005 M $CaCl_2$ and 0.005 $Alcl_3$ was added. NADP was used in the studies of the malic enzyme and of isocitric dehydrogenase. In the former determinations, 0.5 ml of 0.033 M $MgSO_4$, and in the latter, 0.5 ml of 6×10^{-3} M $MnCl_2$ was added. These supplementary solutions were added before the final dilution of the homogenate. The tubes were evacuated for five minutes and were then inverted to add the homogenate contained in the side arm to the mixture in the main portion of the tube, thus bringing the final concentration of homogenate to 0.5 per cent. Following this the tubes were placed in a constant temperature bath at 30°C, and the time required for 90 per cent reduction of methylene blue to occur was determined by visually matching the color with that of a standard tube. This standard contained all of the components of the other tubes except that the methylene blue was diluted to $\frac{1}{10}$ the usual concentration and the homogenate had been previously inactivated by boiling. A control tube containing all the components of the experimental tube except the substrate was used in each determination.

Activities of dehydrogenase enzymes were expressed as 1/time in minutes for 90 per cent decoloration of methylene blue. These activities were determined by subtracting the rate of control from that of the experimental tube.

The activity of cytochrome oxidase, expressed as $\triangle$ log $(CyFe^{++})$/minute, was determined during the same stages of metamorphosis and was measured on tissue homogenates in a final concentration of 1:10,000. The insects were homogenized in 0.03 M phosphate buffer which had a pH of 7.4. The spectrophotometric method of Cooperstein and Lazarow (1951) was used to measure the cytochrome oxidase activity.

TABLE 1. Dehydrogenase activity expressed as 1/time in minutes for 90% decolorization of methylene blue during the metamorphosis of the face fly, *Musca autumnalis*. Readings were made at 30°C. (GPD is alpha-glycerophosphate dehydrogenase.)

	Dehydrogenase									
Stage	Malic	Glu-cose	Alcohol	Lactic	Iso-citric	Glu-tamic	Suc-cinic	GPD I	GPD II	Malic Enzyme
Larva	0.375	0.006	0.055	0.024	0.345	0.008	0.019	0.061	0.005	0.120
Prepupa	0.328	0.004	0.050	0.022	0.316	0.006	0.016	0.050	0.006	0.114
Pupa, 1 day	0.280	0.004	0.040	0.018	0.322	0.006	0.008	0.020	0.008	0.105
Pupa, 2 day	0.252	0.006	0.031	0.015	0.282	0.003	0.008	0.011	0.010	0.082
Pupa, 3 day	0.228	0.010	0.022	0.009	0.230	0.002	0.005	0.005	0.010	0.060
Pupa, 4 day	0.230	0.009	0.038	0.006	0.218	——	0.005	0.004	0.012	0.096
Pupa, 5 day	0.260	0.006	0.046	0.010	0.202	——	0.009	0.018	0.012	0.104
Pupa, 6 day	0.345	0.005	0.058	0.005	0.180	——	0.012	0.029	0.025	0.110
Pupa, 7 day	0.425	0.005	0.062	0.007	0.156	——	0.024	0.058	0.032	0.112
Adult, just emerged	0.785	0.002	0.069	0.007	0.131	——	0.030	0.074	0.035	0.130

OBSERVATIONS

The changes in the activities of the dehydrogenase enzymes during the metamorphosis of the face fly are shown in Table 1. Each value is an average of ten determinations.

Total alpha-glycerophosphate, alcohol, malic, and succinic dehydrogenases as well as the malic enzyme exhibited U-shaped activity. Greatest activity was shown by the malic dehydrogenase with a considerable rise observed in the newly emerged adult. Alpha-glycerophosphate dehydrogenase I (requiring NAD) decreased steadily from the larval stage to the fourth day and then rose gradually during the remainder of the pupal stage. Alpha-glycerophosphate II (not requiring NAD) appeared at the first day of the pupal stage and it showed a steady increase with the highest activity being detected in the adult fly. Isocitric dehydrogenase activity was high initially and remained high until 2-day pupa and thereafter showed a progressive decline. Glucose dehydrogenase activity was very low; it remained fairly steady during the entire pupal stage and decreased slightly in the newly emerged adult. Alcohol dehydrogenase decreased steadily during the first days of the metamorphosis, reaching a low value on the third day, and then increased to reach its highest value in the adult stage. Succinic dehydrogenase exhibited a similar pattern but the level of activity was not as high as most of the other dehydrogenases. The activity of lactic dehydrogenase was low throughout metamorphosis. Malic enzyme exhibited high activities in the larva, prepupa and again in the adult stage. Glutamic dehydrogenase showed low activity in the larval stage. It decreased during the first several days of pupal life and completely disappeared by the fourth day.

Cytochrome oxidase activity was also U-shaped during metamorphosis as indicated in Table 2. Each value here is also an average of at least ten determinations. The larval and prepupal stages were characterized by high activity with a

Table 2. Cytochrome oxidase activity during the metamorphosis of *Musca autumnalis*. Homogenate concentration is 1:10,000.

Stage	Enzyme Activity △ log [$CyFe^{++}$] / min.		
	Minimum	Maximum	Average
Larva	0.051	0.103	0.084
Prepupa	0.043	0.088	0.061
Pupa, 1 day	0.024	0.042	0.032
Pupa, 2 day	0.014	0.038	0.021
Pupa, 3 day	0.009	0.022	0.014
Pupa, 4 day	0.008	0.017	0.010
Pupa, 5 day	0.027	0.048	0.041
Pupa, 6 day	0.052	0.089	0.076
Pupa, 7 day	0.112	0.151	0.127
Adult, newly emerged	0.124	0.221	0.178

progressive decrease until the fourth day and then a steady increase to a high of 0.178.

DISCUSSION

The U-shaped activities of malic dehydrogenase and the malic enzyme agree with the results reported for these enzymes during the metamorphosis of the mealworm and of the house fly (Ludwig and Barsa, 1958 and 1959). These findings also agree with those of Agrell (1949) for the blow fly, *Calliphora erythrocephala*, and of Diamantis (1962) for the Mediterranean flour moth, *Ephestia kühniella*. Isocitric dehydrogenase activity in the face fly was slightly lower than that found in the house fly (Ludwig and Barsa, 1959), but similar in that it uniformly decreased during metamorphosis. This differs from the results reported by Agrell (1949) for the blow fly and Diamantis (1962) for the Mediterranean flour moth, both of whom found that isocitric dehydrogenase exhibited U-shaped activity. Isocitric dehydrogenase in the presence of NADP and Mn^{++} catalyzes the oxidation of isocitrate through oxalosuccinate to alpha-ketoglutarate. The high activity of malic dehydrogenase is similar to that reported for the house fly by Ludwig and Barsa (1959) and for the flour moth by Diamantis (1962). Malic dehydrogenase and the malic enzyme both catalyze the oxidation of l-malate. The end products with the malic enzyme are pyruvate and CO_2, whereas with malic dehydrogenase the end product is oxaloacetate. The high activities of malic dehydrogenase and the malic enzyme coupled with the rather low rate for total lactic dehydrogenase adds additional support to the belief that lactate does not accumulate in insects, but rather pyruvate is reduced to malate which in turn is oxidized to oxaloacetate. The U-shaped activity curves for alcohol and alpha-glycerophosphate I dehydrogenase agree with the results obtained by Ludwig and Barsa (1959) with the house fly, but alpha-glycerophosphate II was found during all stages of metamorphosis in the face fly as contrasted with the house fly where it does not appear until near the end of the

pupal stage. The activity curve for succinic dehydrogenase corroborates reported results of a number of other insects including *Drosophila melanogaster* (Wolsky, 1941), *Calliphora erythrocephala* (Agrell, 1949), *Musca domestica* (Ludwig and Barsa, 1959), *Tenebrio molitor* (Ludwig and Barsa, 1958), *Ephestia kühniella* (Diamantis, 1962). The low activity of this enzyme indicates that it could be a determining factor in the U-shaped respiratory curve that is characteristic of the metamorphosis of holometabolous insects.

The U-shaped pattern of cytochrome oxidase activity here reported for *Musca autumnalis* agrees with what has been found in the fruit fly, *D. melanogaster* by Wolsky (1938), in the house fly, *M. domestica* by Sacktor (1951), in the Japanese beetle, *P. japonica* by Ludwig (1953), in the moth, *Platysamia cecropia* by Williams (1950), and in the flour moth, *Ephestia kühniella* by Diamantis (1912). This would indicate that most of the oxidation during metamorphosis is mediated through the cytochrome system.

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